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Polyparasite Helminth Infections and Their Association to Anaemia and Undernutrition in Northern Rwanda

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Abstract

Background: Intestinal schistosomiasis and soil-transmitted helminth (STH) infections constitute major public health problems in many parts of sub-Saharan Africa. In this study we examined the functional significance of such polyparasite infections in anemia and undernutrition in Rwandan individuals.

Methods: Three polyparasite infection profiles were defined, in addition to a reference profile that consisted of either no infections or low-intensity infection with only one of the focal parasite species. Logistic regression models were applied to data of 1,605 individuals from 6 schools in 2 districts of the Northern Province before chemotherapeutic treatment in order to correctly identify individuals who were at higher odds of being anaemic and/or undernourished.

Findings: Stunted relative to nonstunted, and males compared to females, were found to be at higher odds of being anaemic independently of polyparasite infection profile. The odds of being wasted were 2-fold greater for children with concurrent infection of at least 2 parasites at M+ intensity compared to those children with the reference profile. Males compared to females and anaemic compared to nonanaemic children were significantly more likely to be stunted. None of the three polyparasite infection profiles were found to have significant effects on stunting.

Conclusion: The present data suggest that the levels of polyparasitism, and infection intensities in the Rwandan individuals examined here may be lower as compared to other recent similar epidemiological studies in different regions across sub-Saharan Africa. Neither the odds of anaemia nor the odds of stunting were found to be significantly different in the three-polyparasite infection profiles. However, the odds of wasting were higher in those children with at least two parasites at M+ intensity compared to those children with the reference profile. Nevertheless, despite the low morbidity levels indicated in the population under study here, we recommend sustainable efforts for the deworming of affected populations to be continued in order to support the economic development of the country.

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Introduction

Individuals living primarily in rural areas of low-income countries commonly harbor multiple parasitic infections, including infection with multiple helminth species [1,2,3,4,5,6,7]. In particular, intestinal schistosomiasis and polyparasitic soil-transmitted helminths (STHs) infections constitute major public health problems in sub-Saharan Africa [5,7,8]. Despite the considerable attention in epidemiological literature to the profile of the aforementioned infections, there are very few human studies that have examined the morbidity implications of polyparasitism [9,10,11]. Investigating the implications of polyparasitism morbidity is particularly relevant for healthcare providers in many

developing countries where they must decide screening and treatment strategies in resource-limited settings [10].

The United Nations' fifth report on world nutrition emphasized that malnutrition is the largest contributor to ill-health in the world and that diet-related risk factors for chronic disease are responsible for a large share of the burden of disease in low mortality developing countries [12]. Furthermore, this same report underscored that the effect of such malnutrition is exacerbated by the 4 to 5 billion individuals in the developing world who simultaneously suffer from iron deficiency and its related form of anemia, whilst it also highlighted the high prevalence of anemia throughout the developing world.

The link between hookworm infection and anaemia is well known, and the mechanism of effect through intestinal blood loss

Author Summary

The helminth infections—schistosomiasis, hookworm, ascariasis and trichuriasis—are the main neglected tropical diseases (NTDs) to thrive in sub-Saharan Africa. Here we assess the distribution and the intensities of such polyparasite infections in two districts of the Northern Province in Rwanda and determine whether these are associated with anaemia, lowered haemoglobin levels and recent and/or chronic undernutrition. Rwanda is a small landlocked country in Central Africa where no research or control efforts on NTDs has been conducted since before the genocide in 1994. The current study aimed to elucidate, for the first time post-genocide, the burden of NTDs on the health of the Rwandan people and potential associated morbidity. Despite the fact that we observed low morbidity levels and intensities of polyparasite helminth infections, we recommend sustainable efforts for the deworming of the Rwandan people to be continued in order to offer a worm-free physical and cognitive development to the children of Rwanda and hence support the economic development of the country.

has been described [13,14,15,16,17,18]. Recent large scale studies have suggested links between heavy intensities of *Schistosoma mansoni* infections (the intestinal type of schistosomiasis mainly found in sub-Saharan Africa) with anaemia and lowered haemoglobin counts [19,20,21]. The mechanisms underlying *S. mansoni* associated anaemia are likely multifactorial (e.g., iron deficiency due to extra-corporeal loss, splenic sequestration, autoimmune haemolysis and anaemia of inflammation) and have also been documented [22,23]. Moderate or high intensities of *Trichuris trichiura* are also associated with higher risks of anaemia in the presence of other STHs [24], while the impact of *Ascaris lumbricoides* on anaemia is less clear [10].

Different types of helminth infection may affect nutritional status in different ways (e.g., nutrient absorption, and degree of mucosal damage) [25]. Previous studies indicated various mechanisms through which hookworm, *S. mansoni*, *T. trichiura* and *A. lumbricoides* infections might alter nutritional status [11,22,26,27]. Several studies have found positive associations between malnutrition and the aforementioned intestinal parasites, but they have always limited their focus to single helminth species rather than looking at combinations of helminth species present [28,29,30,31]. In addition, Ezeamama and colleagues [9] have emphasized the lack, and at the same time the need for, epidemiological studies that examine the effect of polyparasite infections at various intensities in a range of morbidities.

In the present study, we have used uniquely detailed data from the Rwandan national Neglected Tropical Disease (NTD) control programme in order to refine and understand the functional significance of polyparasite infections in anaemia and undernutrition in mainly school aged children from two districts in Northern Rwanda. The objectives of this study were to examine the distribution and the intensities of such polyparasite infections as well as to elucidate whether if individuals concurrently infected with multiple helminth species have measurably increased odds of being anaemic and/or undernourished.

Methods

Ethics statement

Ethical approval for Monitoring & Evaluation (M & E) surveys was obtained from the Rwandan National Ethical Committee and

Columbia University's International Review Board. The aim of the survey was explained to the participants, their parents, guardians and teachers before data collection. Moreover, only children who had completed their assent form and presented a consent form signed by their parents were entered in these surveys.

Control programme, study sites, population, sampling, and design

Rwanda is a landlocked country in the Great Lakes region of east-central Africa, bordered by Uganda, Burundi, the Democratic Republic of the Congo and Tanzania. It is one of the smallest countries of Africa (26, 338 km²), but is home to approximately 10.1 million people thus supporting the densest population in continental Africa, with most of the population engaged in subsistence agriculture. A verdant country of fertile and hilly terrain with altitudes varying from 950 m to 4519 m, the small republic bears the title "Land of a Thousand Hills".

The Rwanda MoH through the Centre for Treatment and Research on AIDS, Malaria, Tuberculosis and Other Epidemics (TRAC Plus) - a centre for infectious disease control - was charged with planning and implementing data collection with the assistance from the National Reference Laboratory and the Access project.

For the M & E survey, schools in both districts were randomly selected from three sample frames to allow the programme to be evaluated in 2 low-, 2 medium- and 2 high-schistosomiasis prevalence schools. More precisely these sample frames were defined as follows:

- one school located less than 1 km away from each of the Lakes Ruhondo and Burera- this was aimed to represent a high schistosomiasis prevalence school in each of the 2 districts
- one school located between 2 km and 3 km away from each of the Lakes Ruhondo and Burera- this was aimed to represent a medium schistosomiasis prevalence school in each of the 2 districts
- one school located between 4 km and 5 km away from each of the Lakes Ruhondo and Burera- this was aimed to represent a low schistosomiasis prevalence school in each of the 2 districts

It should be noted that the aforementioned lakes are located in different districts and they were selected on the basis that distance to the lakeshore has been proven useful to screen schools in the greater region [32]. The required sample sizes for children were calculated based on schistosomiasis prevalence/intensity data from schools in various African countries with similar age ranges assuming expected reductions in *S. mansoni* intensities over two annual treatments through EpiSchisto software (<http://www.schoolsandhealth.org/epidynamics.htm>); more technical details have been described elsewhere [33] and hence they are not repeated here. In addition, 120 adults were randomly selected in two villages from the two aforementioned districts, which were located less than 1 km away from each of the lakes. This adult subgroup was included with the aim of monitoring the future impact of Mass Drug Administration (MDA) on *S. mansoni*-related hepatic fibrosis, where highest morbidity/symptomatology tends to be displayed in this older age group. However, for the purposes of the current analyses, we decided to include data from individuals of up to 20 years old, thereby inclusive of the end of the growing period for late maturers.

These data were collected during February to April in 2008, based on results of mapping surveys in 2007 (data not presented here) with the aim to determine pre-treatment levels of the infection status and some clinical indicators.

Infection intensity

The parasite burden was determined by duplicate examination from different microscopists of one stool specimen, at the same time, from each study participant for the presence of *S. mansoni*, *T. trichiura*, *A. lumbricoides* and hookworm (*Ancylostoma duodenale*) by the Kato-Katz method. This was due to logistical and financial reasons and can be justified within the scale of a large-scale control programme, although we are fully aware that replicate stool samples over several days are ideally required to accurately estimate intensity of schistosomiasis and STH. The mean number of eggs per gram (EPG) of stool for each parasite was used to define infections of low and moderate/high (M+) intensity in accordance with WHO-established intensity cutoff values for *S. mansoni*, *T. trichiura*, hookworm and *A. lumbricoides* infections.

Primary determinant: parasite infection profiles

Parasite infection profiles were based on infection status of the study participants; these parasite infection profiles were created using a similar technique developed in a study conducted in rice-farming villages in Leyte, The Philippines [9].

Given possible concurrent infection by up to four parasites at one of three potential intensity levels (none, low, or M+) for each species, there were $3^4 = 81$ possible unique categories of polyparasite infections. A total of 47 of the 81 categories were found in the current Rwandese study population. The sub profiles were finally condensed into the following 4 infection profiles corresponding to putatively different risk levels for anaemia and undernutrition:

1. Reference profile (n = 189): no infection or infection with 1 parasite species at low intensity;
2. Polyparasite infection profile I (n = 582): concurrent infection with 2, 3, or 4 parasite species at low intensity;
3. Polyparasite infection profile II (n = 543): infection with 1 parasite species at M+ intensity and all other parasite species present at low intensity or absent;
4. Polyparasite infection profile III (n = 291): concurrent infection with at least 2 parasite species at M+ intensity and all other parasite species present at low intensity or absent. More precisely, in this polyparasite infection profile 6 children had concurrent infection with 3 or 4 parasites at M+ intensity.

Morbidity indicators

Heights were measured with height poles which had a fixed head board and can thus be considered comparable to that of the NHANES stadiometer (http://www.cdc.gov/nchs/products/elec_prods/subject/video.htm). More precisely, the stature meter was placed to the floor and for each individual the tape was pulled up until zero reached the red line. The upper part of the pole was then firmly and accurately attached to the wall and fixed with screws. Finally the meter was pulled down onto the head of individual to get the measurement. Weights were measured with electronic balances. Children were asked to remove their shoes and all heavy clothes if they wore any and this was done in the morning by the survey team. All persons performing these measurements were fully trained and experienced in the use of these protocols, and the same staffs were used throughout to ensure standardization.

Finger prick blood samples were also obtained from each individual, sufficient for accurate Hb measurement using a Hemocue photometer [34]. Indices of the anthropometric status of the studied children were based on the 2000 growth reference

curves designed by the Centre for Disease Control (CDC) as this population more closely resembles those in countries like Rwanda since it includes both human milk and formula-fed infants; these were computed using the Nutstat program within Epi Info V 3.4. The fact that the 2000 CDC growth charts consist of sex specific charts for infants, birth to age 36 months (length-for-age, weight-for-length, weight-for-age, and head circumference-for-age) and older children, 2 to 20 years (stature-for-age, weight-for-age and Body Mass Index (BMI)-for-age) led us also to the decision of excluding data of individuals more than 20 years old. Low Body Mass Index is considered an indicator of acute under-nutrition (thinness or wasting) and is generally associated with failure to gain weight or a loss of weight [35]. The Z-score cut-off point recommended by WHO, CDC, and others to classify low anthropometric levels is 2 Standard Deviation (SD) units below the reference median for this specific index. A cut-off of -2 BMI Z-scores was calculated to classify underweight individuals. The z-scores of height-for-age that were less than 2 SD below the reference median served to define stunted individuals.

Statistical methods

In order to examine the adjusted odds ratios (ORs) of anaemia, wasting and stunting, we tested a range of different approaches of statistical modeling to correctly identify individuals who have had higher morbidity as assessed from the outcomes aforementioned. Because the modeling of the between school variation through random effects logistic regressions did not prove appropriate for the statistical analysis of our data, we also employed the Generalized Estimating Equations (GEE) approach whenever this was analytically possible. If the GEE algorithm did not converge, we used conventional logistic regression models. The GEE method does not explicitly model between-cluster variation; instead it focuses on and it estimates its counterpart, the within-cluster similarity of the residuals; it then uses this estimated correlation to reestimate the regression parameters and to calculate standard errors which are reasonably accurate and hence lead to the generation of confidence intervals with the correct coverage rates [36]. Data management and statistical analyses were performed using SAS V9 (SAS Institute Inc., Cary, NC, USA).

For all the odds ratios studied, we fitted the random effects logistic regression models by using PROC NLMIXED while we employed the GEE method by using PROC GENMOD. Particularly for the odds of anaemia, we have included as explanatory variable the parasite infection profiles I-III (as defined in the previous section); we also consider the nutritional status as defined by stunting as an effect modifier. We therefore display estimates with and without considering the effect of stunting; we also included the interaction term of stunting with the parasite infection profiles if the change in deviances between relevant nested models was significant at the 5% significance level. Similarly, when we modeled the odds of wasting and stunting respectively, we have included as explanatory variable the parasite infection profiles I-III while we consider anaemia status as an effect modifier. Potential confounders of the relationships between anaemia stunting, wasting and helminth infection were decided to be included in light of known confounders of these associations based on published literature [11,20,21,37,38,39] and these were the categories of age, sex, and the district where study participants were living in.

Mean Hb concentration of different groups of individuals recruited in the current study were also initially examined through a random effects at the school level multivariate linear regression model by using PROC MIXED. Likelihood ratio tests indicated that these random effects were not significant and thus it was

finally decided to omit them. However, because of the non-independence found in our data we finally decided to employ the GEE method by using PROC GENMOD. We tested as explanatory variables the categories of age, sex, district and parasite infection profiles I–III. We also tested the two-way interaction terms of parasite infection profiles I–III with district and stunting and retained them in the model if the change in deviances between the relevant nested models was significant at the 5% significance level.

Covariates in all aforementioned multivariable models with $p < 0.05$ were considered significantly associated with outcomes.

Results

A total of 1605 children and adolescents were recruited, for a participation rate of 88%, and provided complete parasitologic and anthropometric data. They were aged 5 to 20 years old, with a median age of 10 years and 47.7% of the recruited individuals were male. The observed prevalences of wasting, stunting and anaemia were respectively estimated as following: 8.1% (95% CI: 6.8 to 9.4), 38.5% (36.1 to 40.9) and 4.9% (95% CI: 3.9 to 6.1). The mean observed Hb concentration was estimated to be 13.8 g/dL (95% CI: 13.7 to 13.8).

Table 1 contains the characteristics of the study population by subprofile classification infection category. The most prevalent co-infections were those of low intensity of *A. lumbricoides* and *T. trichiura* (21.2%).

The adjusted ORs of anaemia from the GEE multivariate logistic regression models are presented in Table 2. Deviance tests indicated 'Model 3' as the most appropriate one; this model shows that individuals who are stunted are almost 1.5 times more likely than non-stunted to be anaemic (OR: 1.6, $P = 0.041$). Children of 11–13 years old were significantly less likely than children of 5–7 years old to be anaemic (OR = 0.572, $P = 0.026$). In addition, males are almost twice more likely to be anaemic compared to females (OR: 1.9, $P = 0.024$). Neither the interaction terms of stunting or district with the parasite infection profiles nor any other examined variable here were found to be significant factors for the odds of being anaemic.

GEE did not converge for the modeling of the odds of being wasted and this is most likely to be explicable by the fact that there was not sufficient information in order to estimate the binomial probability structure by taking into account the intra-subject correlation. Consequently, we used Maximum Likelihood (ML) and the results of such multivariate logistic regression models for the odds of being wasted are presented in Table 3. Deviance tests as well as Akaike's information criterion (AIC) indicated 'Model 2' as the best one among the tested models. This model shows that only children of 11–13 years old were significantly more likely than the younger children (age group: 5–7 years old) to be wasted (OR = 1.8, $P = 0.033$). Furthermore, study participants from Burera district were significantly more likely to be wasted when compared with study participants from Musanze district (OR = 3.3, $P < 0.001$). It is noteworthy that children with concurrent infection of at least 2 parasite species at M+ intensity – that is, those with polyparasite infection profiles III – were almost twice marginally significantly more likely to be wasted than children with the reference polyparasite infection profile (OR = 2.2, $P = 0.054$). Neither the interaction terms of anaemia status or district with the parasite infection profiles nor any other examined variable here, were found to be significant factors for the odds of being wasted.

Table 4 contains the results from the GEE multivariate logistic regression models for the odds of being stunted. Deviance tests

indicated 'Model 3' as the most appropriate one; this model shows those children of 11–17 years old to have significant positive ORs if compared with the youngest age group examined here (i.e. 5–7 years old), (more specifically, 11–13 years old: OR = 2.4, $P = 0.001$; 14–17 years old: OR = 1.4, $P = 0.044$). However, adolescents of 18–20 years old were significantly less likely than the youngest age group to be stunted (OR = 0.4, $P = 0.003$). Male individuals were significantly more likely than females to be stunted (OR = 1.9, $P < 0.001$). Study participants from Burera district were significantly less likely to be stunted than the study participants from Musanze district (OR = 0.4, $P < 0.001$). Anaemic study participants were significantly more likely than non anaemic to be stunted (OR = 1.7, $P = 0.020$). Neither the interaction terms of anaemia status or district with the parasite infection profiles nor any other examined variable here were found to be significant factors for the odds of being stunted.

Finally, Table 5 contains the results from the GEE linear regression model for the mean Hb concentration and the mean differences in different groups of the study population here. Deviance tests indicated 'Model 3' as the most appropriate one; this model shows that on average Hb concentration in the study population was 13.109 g/dL (95% CI: 12.904–13.314). All different categories of age yielded significant associations with increased Hb levels compared to the youngest age group examined here (i.e., 5–7 years old). Study participants who were stunted when compared to non-stunted had significantly lower Hb counts by 0.270 g/dL, respectively ($P < 0.001$).

Discussion

Concurrent multiple parasite infections were found to be the norm in our study population, as has been reported in studies published elsewhere [1,2,5,6,9,10]. However, in the current study population, none of the concurrent polyparasite infections were found to be significantly associated with higher odds of anaemia, wasting, stunting nor mean lowered Hb concentration. Nevertheless, results did indicate that those study participants with concurrent infection with at least 2 parasites at M+ intensity were marginally significantly more likely to be wasted ($P = 0.054$) relative to those with no infection or infection with 1 parasite species at low intensity, thereby validating the impact of higher intensity infections on health [40].

Potential reasons for the general lack of association of the concurrent polyparasite infections with anaemia in the current Rwandan population might be that anaemia itself appears to be relatively uncommon in this area. One reason for the latter may relate also to the fact that malaria incidence in the two districts studied here is lower than the rest of the country, as well as to the decrease of malaria prevalence in Rwanda in general as an unpublished WHO Draft of Mid Term Evaluation Report of the Rwandan Malaria Strategic Plan 2005–2010, reveals. In addition, as Table 1 indicates, the majority of the study participants (i.e. 21.2%) had low intensities of *A. lumbricoides* and *T. trichiura* while very few of them had M+ intensities of hookworm and *S. mansoni* infections. Such a distribution is likely to have limited the power of this study – potentially making it difficult to achieve statistical significance where one existed for co-infections of M+ intensities. M+ intensities of the latter two helminth infections have been recently shown to be significant factors for anaemia in other similar epidemiological studies [20,21], and such combined findings highlight how different factors contribute to anaemia in different parasite transmission and eco-epidemiological settings. Indeed we would recommend further similar studies in the eastern part of Rwanda where there is a higher prevalence of hookworm

Table 1. Characteristics of the study population.

Subprofile classification, infection category	Children No (%)	Mean hemoglobin level, g/dL	Individuals with anemia ^a No (%) ^b	Stunted Individuals ^c No (%) ^d	Wasted Individuals ^e No (%) ^f
Subprofile 0: 0 or 1 L infection (n = 189)					
0 infections	31 (1.93)	13.72	1 (3.23)	12 (38.71)	1 (3.23)
<i>S. mansoni</i>	4 (0.25)	14.20	0 (0.00)	2 (50.00)	0 (0.00)
Hookworm	12 (0.75)	13.58	1 (8.33)	4 (33.33)	1 (8.33)
<i>T. trichiura</i>	97 (6.04)	13.64	5 (5.15)	34 (35.05)	1 (1.03)
<i>A. lumbricoides</i>	45 (2.80)	13.81	4 (8.89)	16 (35.56)	5 (11.11)
Subprofile 1: 2 L infections (n = 404)					
<i>S. mansoni</i> and Hookworm	2 (0.12)	14.30	0 (0.00)	2 (100.00)	0 (0.00)
<i>S. mansoni</i> and <i>A. lumbricoides</i>	10 (0.62)	14.26	0 (0.00)	3 (30.00)	2 (20.00)
<i>S. mansoni</i> and <i>T. trichiura</i>	14 (0.87)	13.99	0 (0.00)	7 (50.00)	2 (14.29)
<i>T. trichiura</i> and Hookworm	28 (1.74)	14.32	2 (7.14)	12 (42.86)	2 (7.14)
<i>A. lumbricoides</i> and Hookworm	10 (0.62)	13.75	0 (0.00)	6 (60.00)	1 (10.00)
<i>A. lumbricoides</i> and <i>T. trichiura</i>	340 (21.18)	13.74	16 (4.71)	121 (35.59)	26 (7.65)
Subprofile 2: 3 L infections (n = 157)					
<i>S. mansoni</i> , Hookworm and <i>T. trichiura</i>	4 (0.25)	15.25	0 (0.00)	1 (25.00)	0 (0.00)
<i>S. mansoni</i> , Hookworm and <i>A. lumbricoides</i>	2 (0.12)	13.90	0 (0.00)	0 (0.00)	0 (0.00)
<i>S. mansoni</i> , <i>A. lumbricoides</i> and <i>T. trichiura</i>	54 (3.36)	14.01	2 (3.70)	20 (37.04)	2 (3.70)
<i>A. lumbricoides</i> , Hookworm and <i>T. trichiura</i>	97 (6.04)	13.91	6 (6.19)	48 (49.48)	2 (2.06)
Subprofile 3: 4 L infections (n = 21)					
<i>A. lumbricoides</i> , Hookworm, <i>S. mansoni</i> and <i>T. trichiura</i>	21 (1.31)	14.16	1 (4.76)	9 (42.86)	2 (9.52)
Subprofile 4: 1 M+ infection (n = 25)					
<i>S. mansoni</i>	2 (0.12)	14.50	0 (0.00)	1 (50.00)	0 (0.00)
<i>T. trichiura</i>	11 (0.69)	13.81	0 (0.00)	4 (36.36)	2 (18.18)
<i>A. lumbricoides</i>	12 (0.75)	13.62	0 (0.00)	6 (50.00)	1 (8.33)
Subprofile 5: 1 M+ infection and 1 L infections (n = 337)					
<i>S. mansoni</i> (M+) and Hookworm (L)	2 (0.12)	15.75	0 (0.00)	2 (100.00)	0 (0.00)
<i>S. mansoni</i> (M+) and <i>T. trichiura</i> (L)	2 (0.12)	14.20	0 (0.00)	1 (33.33)	0 (0.00)
<i>S. mansoni</i> (M+) and <i>A. lumbricoides</i> (L)	4 (0.25)	14.23	0 (0.00)	1 (25.00)	0 (0.00)
<i>T. trichiura</i> (M+) and <i>A. lumbricoides</i> (L)	47 (2.93)	13.57	2 (4.26)	12 (25.53)	7 (14.89)
<i>T. trichiura</i> (M+) and <i>S. mansoni</i> (L)	1 (0.06)	14.10	0 (0.00)	1 (100.00)	1 (100.00)
<i>T. trichiura</i> (M+) and Hookworm (L)	1 (0.06)	14.70	0 (0.00)	0 (0.00)	0 (0.00)
<i>A. lumbricoides</i> (M+) and Hookworm (L)	7 (0.44)	14.26	0 (0.00)	1 (14.29)	0 (0.00)
<i>A. lumbricoides</i> (M+) and <i>T. trichiura</i> (L)	273 (17.01)	13.58	16 (5.86)	102 (37.36)	24 (8.79)
Subprofile 6: 1 M+ infection and 2 L infections (n = 149)					
<i>S. mansoni</i> (M+), Hookworm (L) and <i>T. trichiura</i> (L)	5 (0.31)	14.50	0 (0.00)	2 (40.00)	0 (0.00)
<i>S. mansoni</i> (M+), <i>A. lumbricoides</i> (L) and <i>T. trichiura</i> (L)	18 (1.12)	14.50	1 (5.56)	9 (50.00)	2 (11.11)
<i>T. trichiura</i> (M+), Hookworm (L) and <i>A. lumbricoides</i> (L)	15 (0.93)	14.09	0 (0.00)	9 (60.00)	1 (6.67)
<i>T. trichiura</i> (M+), <i>S. mansoni</i> (L) and <i>A. lumbricoides</i> (L)	4 (0.25)	13.40	0 (0.00)	2 (50.00)	1 (25.00)
<i>T. trichiura</i> (M+), <i>S. mansoni</i> (L) and Hookworm (L)	1 (0.06)	10.40	1 (100.00)	0 (0.00)	0 (0.00)
<i>A. lumbricoides</i> (M+), Hookworm (L) and <i>T. trichiura</i> (L)	81 (5.05)	13.84	5 (6.17)	37 (45.68)	5 (6.17)
<i>A. lumbricoides</i> (M+), <i>S. mansoni</i> (L) and <i>T. trichiura</i> (L)	25 (1.56)	13.96	3 (12.00)	14 (56.00)	2 (8.00)
Subprofile 7: 1 M+ infection and 3 L infections (n = 32)					
<i>S. mansoni</i> (M+), Hookworm (L), <i>A. lumbricoides</i> (L) and <i>T. trichiura</i> (L)	20 (1.25)	13.92	1 (5.00)	12 (60.00)	0 (0.00)
<i>A. lumbricoides</i> (M+), <i>S. mansoni</i> (L), Hookworm (L) and <i>T. trichiura</i> (L)	12 (0.75)	14.33	0 (0.00)	3 (25.00)	1 (8.33)
Subprofile 8: 2 M+ infections (n = 201)					
<i>A. lumbricoides</i> (M+) and <i>T. trichiura</i> (M+)	201 (12.52)	13.63	10 (4.98)	74 (36.82)	33 (16.42)
Subprofile 9: 2 M+ and 1 L infections (n = 64)					
<i>S. mansoni</i> (M+), <i>T. trichiura</i> (M+) and <i>A. lumbricoides</i> (L)	2 (0.12)	12.05	1 (50.00)	1 (50.00)	0 (0.00)

Table 1. Cont.

Subprofile classification, infection category	Children No (%)	Mean hemoglobin level, g/dL	Individuals with anemia ^a No (%) ^b	Stunted Individuals ^c No (%) ^d	Wasted Individuals ^e No (%) ^f
<i>S. mansoni</i> (M+), <i>A. lumbricoides</i> (M+) and <i>T. trichiura</i> (L)	17 (1.06)	14.29	1 (5.26)	5 (29.41)	0 (0.00)
<i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (M+) and <i>S. mansoni</i> (L)	15 (0.93)	13.42	0 (0.00)	7 (46.67)	2 (13.33)
<i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (M+) and Hookworm (L)	29 (1.81)	13.27	1 (3.45)	8 (27.59)	1 (3.45)
<i>A. lumbricoides</i> (M+), <i>S. mansoni</i> (M+) and <i>T. trichiura</i> (L)	1 (0.06)	14.60	0 (0.00)	0 (0.00)	0 (0.00)
Subprofile 10: 2 M+ and 2 L infections (n = 20)					
<i>S. mansoni</i> (M+), <i>T. trichiura</i> (M+), Hookworm (L) and <i>A. lumbricoides</i> (L)	2 (0.12)	14.65	0 (0.00)	1 (50.00)	0 (0.00)
<i>S. mansoni</i> (M+), <i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (L) and Hookworm (L)	7 (0.44)	14.49	0 (0.00)	0 (0.00)	0 (0.00)
<i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (M+), Hookworm (L) and <i>S. mansoni</i> (L)	11 (0.69)	14.35	0 (0.00)	6 (54.55)	0 (0.00)
Subprofile 11: 3 M+ infections or 3 M+ and 1 L infections (n = 6)					
<i>S. mansoni</i> (M+), <i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (M+)	2 (0.12)	11.80	1 (50.00)	1 (50.00)	0 (0.00)
<i>S. mansoni</i> (M+), <i>A. lumbricoides</i> (M+), <i>T. trichiura</i> (M+) and Hookworm (L)	4 (0.25)	12.85	0 (0.00)	0 (0.00)	0 (0.00)

^aAnaemia was defined for all tables displayed (according to WHO guidelines), as Hb less than 11.5 g/dL for children from 5 to 11 years old and for children between 12 and 14 years old as Hb less than 12.0 g/dL. For individuals aged more than 14 years old, anaemia was defined as Hb less 12.0 g/dL for females and Hb less than 13.0 g/dL for males.

^bPercentages in this column denote percentages of anaemic within each specific subprofile classification/infection category.

^cStunting was defined as height for age z-score (HAZ) less than -2.

^dPercentages in this column denote percentages of stunted within each specific subprofile classification/infection category.

^eWasting was defined as body mass index z-score (BMIZ) less than -2.

^fPercentages in this column denote percentages of wasted within each specific subprofile classification/infection category.

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as shown by the STH mapping survey conducted last year by the NTD control programme (unpublished data) and malaria together with other country and epidemiological settings, to further elucidate the potential association of polyparasitism to human morbidity.

The present study also indicated children of 11–13 years old to be significantly less likely than children of 5–7 years old to be anaemic. This finding might be explained by the fact that the youngest children have recently experienced the high iron demand of early childhood. We also found males compared to females to be significantly more likely to be anaemic. A previous study has discussed that among younger children, boys are more anaemic than girls but the reasons for this remain still unknown [41]. We have also attempted to examine if the differences between the sexes in the odds of being anaemic varied by age, but when the statistical interactions of age and sex were included in the relevant model, the algorithm did not converge. Stunted children compared to non stunted were also demonstrated to be more likely to be anaemic. This result is supported by the observation that iron deficiency which leads to anaemia also contributes to poor growth while it has been demonstrated that supplementation of iron to anemic children has a positive effect on linear growth [42]. The effect of the polyparasite infection profile on anaemia was not found to vary according to stunting.

We also assessed the association between the polyparasite infection profiles and acute under-nutrition. Children with concurrent infection with at least two parasites at M+ intensity relative to those with no infection or infection were found to be marginally significantly more likely to be wasted ($P = 0.054$). This finding might be explained by decreased appetite experienced in those individuals who harbored two or more parasites at M+ intensity. However, significant differences in the odds of wasting observed within the two districts studied here still remain unclear

as we do not think that the latter would differ in dietary patterns or socio-economic status.

Regarding chronic undernutrition and concurrent polyparasite infections, the present cross-sectional study did not find any significant association with the exception of age and anaemia status being revealed as a significant factor for stunting. Older individuals, with the exception of the age group of 18–20 years old, were found to be more likely to be stunted than the younger age group studied here (i.e. 5–7 years old). This could imply prior malnutrition in these individuals as has previously been reported in Zanzibar and Burkina Faso [43,44]. Furthermore, the findings of decreased odds of stunting in the older age of 18–20 years old suggest compensatory growth in height for this age group and this is consistent with results from longitudinal Senegalese data [45]. Anaemic compared to non-anaemic individuals were also found to be significantly more likely to be stunted and the causal pathways for such results have been discussed in the previous paragraph. However, the effect of the polyparasite infection profile on stunting was not found to vary according to anaemia status. Finally, it should be noted that stunting - an indicator of chronic undernutrition - was the most prevalent form of undernutrition observed in this study. This has been also found by the Rwanda demographic health survey 2005 where the Northern province had the highest prevalence of severe chronic malnutrition [46]. Nevertheless, for the same reasons as mentioned above, any explanation for the significant differences in the odds of stunting observed within the two districts studied here still remain unclear.

Our investigation has some limitations. As mentioned above, due in part to the overdispersed nature of helminth infections eggs in stool and daily variation in excretion, the ideal protocol is to use replicate faecal samples over several (ideally a minimum of three) consecutive days [47]. Unfortunately due to the logistical and financial constraints inherent within the scale of such a large-scale

Table 2. Adjusted ORs from GEE multivariate logistic regression model of anaemia (n = 1605).

Variable	Categories	Adjusted ORs (95% CI) [○]	p-values
Model 1 [†] , Deviance = 623.193, DF = 1596			
Age	5–7 years	1	
	8–10 years	0.647 (0.381–1.099)	0.107
	11–13 years	0.631 (0.385–1.034)	0.068
	14–17 years	0.759 (0.408–1.411)	0.383
	18–20 years	1.629 (0.732–3.622)	0.232
Sex	Female	1	
	Male	2.022 (1.145–3.569)	0.015
Polyparasite infection profile [*]	Reference	1	
	I	0.793 (0.499–1.258)	0.324
	II	0.877 (0.605–1.271)	0.489
	III	0.733 (0.445–1.209)	0.224
Model 2 [‡] , Deviance = 622.259, DF = 1595			
Age	5–7 years	1	
	8–10 years	0.640 (0.373–1.099)	0.106
	11–13 years	0.621 (0.383–1.006)	0.053
	14–17 years	0.747 (0.411–1.357)	0.338
	18–20 years	1.682 (0.662–4.276)	0.275
Sex	Female	1	
	Male	2.018 (1.140–3.571)	0.016
Polyparasite infection profile	Reference	1	
	I	0.799 (0.495–1.290)	0.359
	II	0.855 (0.614–1.191)	0.354
	III	0.694 (0.436–1.107)	0.101
District	Musanze	1	
	Burera	1.258 (0.826–1.916)	0.650
Model 3 [◇] , Deviance = 619.540, DF = 1595			
Age	5–7 years	1	
	8–10 years	0.619 (0.367–1.043)	0.072
	11–13 years	0.572 (0.350–0.935)	0.026
	14–17 years	0.715 (0.405–1.263)	0.248
	18–20 years	1.674 (0.784–3.574)	0.183
Sex	Female	1	
	Male	1.883 (1.086–3.265)	0.024
Polyparasite infection profile	Reference	1	
	I	0.788 (0.501–1.238)	0.300
	II	0.863 (0.607–1.228)	0.413
	III	0.739 (0.449–1.217)	0.235
Stunting	Non stunted	1	
	Stunted	1.590 (1.018–2.484)	0.041

[○] 95% CIs are based on empirical standard errors.

[†] Model 1 included as explanatory variables the categories of age, sex and polyparasite infection profiles.

^{*} Definitions for polyparasite profiles have been described in the 'Methods' section.

[‡] Model 2 included as explanatory variables the categories of age, sex, polyparasite infection profiles and district.

[◇] Model 3 included as explanatory variables the categories of age, sex, polyparasite infection profiles, and stunting.

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Table 3. Adjusted odds ratios from ML multivariate logistic regression model of wasting (n = 1605).

Variable	Categories	Adjusted ORs (95% CI)	p-values
Model 1 [†] , Deviance = 880.568, AIC = 898.568, DF = 1596			
Age	5–7 years	1	
	8–10 years	0.995 (0.593–1.671)	0.985
	11–13 years	1.831 (1.061–3.161)	0.030
	14–17 years	0.986 (0.573–1.697)	0.959
	18–20 years	NA	NA
Sex	Female	1	
	Male	0.946 (0.658–1.359)	0.763
Polyparasite infection profile [*]	Reference	1	
	I	1.575 (0.721–3.442)	0.255
	II	2.093 (0.969–4.522)	0.060
	III	3.081 (1.396–6.800)	0.005
Model 2 [‡] , Deviance = 846.862, AIC = 866.862, DF = 1595			
Age	5–7 years	1	
	8–10 years	1.028 (0.609–1.736)	0.917
	11–13 years	1.824 (1.050–3.170)	0.033
	14–17 years	0.975 (0.564–1.684)	0.927
	18–20 years	NA	NA
Sex	Female	1	
	Male	0.973 (0.674–1.403)	0.882
Polyparasite infection profile	Reference	1	
	I	1.617 (0.735–3.556)	0.232
	II	1.779 (0.817–3.872)	0.147
	III	2.206 (0.988–4.928)	0.054
District	Musanze	1	
	Burera	3.264 (2.136–4.990)	<0.001
Model 3 [◇] , Deviance = 846.366, AIC = 868.366, DF = 1594			
Age	5–7 years	1	
	8–10 years	1.023 (0.606–1.727)	0.932
	11–13 years	1.808 (1.040–3.144)	0.036
	14–17 years	0.968 (0.560–1.673)	0.907
	18–20 years	NA	NA
Sex	Female	1	
	Male	0.967 (0.670–1.395)	0.858
Polyparasite infection profile	Reference	1	
	I	1.604 (0.729–3.530)	0.240
	II	1.775 (0.816–3.865)	0.148
	III	2.195 (0.983–4.905)	0.055
District	Musanze	1	
	Burera	3.271 (2.140–5.000)	<0.001
Anaemia	Non Anaemic	1	
	Anaemic	0.724 (0.283–1.849)	0.499

[†] Model 1 included as explanatory variables the categories of age, sex and polyparasite infection profiles.

[‡] NA stands for 'Not Available' as there were no individuals of 18–20 years old who were wasted and thus the model did not provide any estimates for this specific category.

^{*} Definitions for polyparasite profiles have been described in the 'Methods' section.

[‡] Model 2 included as explanatory variables the categories of age, sex, polyparasite infection profiles and district.

[◇] Model 3 included as explanatory variables the categories of age, sex, polyparasite infection profiles, and district and anaemia status.

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Table 4. Adjusted odds ratios from GEE multivariate logistic regression model of stunting (n = 1605).

Variable	Categories	Adjusted ORs (95% CI) ○	p-values
Model 1 [†] , Deviance = 2073.436, DF = 1596			
Age	5–7 years	1	
	8–10 years	1.390 (0.937–2.062)	0.102
	11–13 years	2.237 (1.370–3.654)	0.001
	14–17 years	1.337 (0.953–1.875)	0.092
	18–20 years	0.360 (0.143–0.909)	0.031
Sex	Female	1	
	Male	1.904 (1.648–2.200)	<0.001
Polyparasite infection profile [*]	Reference	1	
	I	1.030 (0.742–1.431)	0.858
	II	1.249 (0.849–1.837)	0.259
	III	1.136 (0.642–2.010)	0.662
Model 2 [‡] , Deviance = 2016.559, DF = 1595			
Age	5–7 years	1	
	8–10 years	1.450 (0.963–2.182)	0.075
	11–13 years	2.354 (1.414–3.919)	0.001
	14–17 years	1.403 (0.998–1.972)	0.051
	18–20 years	0.405 (0.214–0.769)	0.006
Sex	Female	1	
	Male	1.973 (1.700–2.289)	<0.001
Polyparasite infection profile	Reference	1	
	I	1.043 (0.747–1.456)	0.806
	II	1.280 (0.872–1.880)	0.208
	III	1.189 (0.660–2.142)	0.565
District	Musanze	1	
	Burera	0.427 (0.309–0.588)	<0.001
Model 3 [◇] , Deviance = 2012.140, DF = 1594			
Age	5–7 years	1	
	8–10 years	1.465 (0.973–2.206)	0.068
	11–13 years	2.384 (1.428–3.981)	0.001
	14–17 years	1.412 (1.009–1.977)	0.044
	18–20 years	0.397 (0.214–0.735)	0.003
Sex	Female	1	
	Male	1.942 (1.692–2.228)	<0.001
Polyparasite infection profile	Reference	1	
	I	1.049 (0.755–1.458)	0.775
	II	1.286 (0.884–1.871)	0.189
	III	1.201 (0.672–2.146)	0.536
District	Musanze	1	
	Burera	0.424 (0.307–0.585)	<0.001
Anaemia	Non Anaemic	1	
	Anaemic	1.671 (1.086–2.572)	0.020

○ 95% CIs are based on empirical standard errors.

[†]Model 1 included as explanatory variables the categories of age, sex and polyparasite infection profiles.^{*}Definitions for polyparasite profiles have been described in the 'Methods' section.[‡]Model 2 included as explanatory variables the categories of age, sex, polyparasite infection profiles and district.[◇]Model 3 included as explanatory variables the categories of age, sex, polyparasite infection profiles, and district and anaemia status.

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Table 5. Estimated differences in mean Hb concentration at baseline for the effects of selected explanatory variables from a GEE linear regression model (n = 1605).

Variable	Categories	Adjusted mean differences (95% CI) ○	p-values
Model 1 [†] , Deviance = 2385.795, DF = 1596			
Age	Intercept (5–7 years, female, reference polyparasite profile)	13.052 (12.859–13.246)	<0.001
	8–10 years	0.473 (0.391–0.556)	<0.001
	11–13 years	0.845 (0.679–1.012)	<0.001
	14–17 years	1.342 (1.113–1.571)	<0.001
	18–20 years	1.628 (1.137–2.120)	<0.001
Sex	Male	–0.100 (–0.227–0.027)	0.124
Polyparasite infection profile [*]	I	0.093 (–0.131–0.317)	0.418
	II	0.079 (–0.167–0.325)	0.530
	III	0.052 (–0.145–0.248)	0.605
Model 2 [‡] , Deviance = 2381.176, DF = 1595			
Age	Intercept (5–7 years, female, reference polyparasite profile, Musanze)	13.098 (12.877–13.319)	<0.001
	8–10 years	0.471 (0.392–0.550)	<0.001
	11–13 years	0.845 (0.681–1.010)	<0.001
	14–17 years	1.342 (1.115–1.568)	<0.001
	18–20 years	1.631 (1.154–2.109)	<0.001
Sex	Male	–0.099 (–0.229–0.031)	0.135
Polyparasite infection profile	I	0.090 (–0.135–0.315)	0.434
	II	0.081 (–0.169–0.330)	0.527
	III	0.057 (–0.154–0.268)	0.597
District	Burera	–0.091 (–0.352–0.171)	0.497
Model 3 [◇] , Deviance = 2361.793, DF = 1595			
Age	Intercept (5–7 years, female, reference polyparasite profile, non stunted)	13.109 (12.904–13.314)	<0.001
	8–10 years	0.494 (0.426–0.561)	<0.001
	11–13 years	0.896 (0.755–1.037)	<0.001
	14–17 years	1.360 (1.142–1.577)	<0.001
	18–20 years	1.583 (1.081–2.085)	<0.001
Sex	Male	–0.060 (–0.181–0.061)	0.330
Polyparasite infection profile	I	0.096 (–0.139–0.330)	0.424
	II	0.092 (–0.155–0.340)	0.464
	III	0.060 (–0.155–0.274)	0.587
Stunting	Stunted	–0.270 (–0.336–0.205)	<0.001

○ 95 % CIs are based on empirical standard errors.

[†]Model 1 included as explanatory variables the categories of age, sex and polyparasite infection profiles.^{*}Definitions for polyparasite profiles have been described in the 'Methods' section.[‡]Model 2 included as explanatory variables the categories of age, sex, polyparasite infection profiles and district.[◇]Model 3 included as explanatory variables the categories of age, sex, polyparasite infection profiles, and stunting.

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control programme, such ideals cannot realistically be met and hence duplicate Kato Katz thick smears were taken from a single day's stool per individual instead. We are aware that such an assessment method is likely to have introduced some misclassification in the measurement of the intensities of helminth infections and consequently in the allocation of study participants to the polyparasite infection profiles. In addition, although we do recognize that anthropometric measurements should be taken according to the standardized protocols used by NHANES to develop the growth charts, some modification to these gold standard measures are necessarily within the field conditions of Mass Drug Administration. Nevertheless, we are confident that every possible precaution was employed by the Rwanda field team in order to obtain accurate and high quality reproducible data. Furthermore, we believe that it is highly unlikely that the examined associations of this study were biased by unmeasured confounding factors such as socio-economic status of the study participants despite the fact that helminth infections are known to be intimately linked with poverty [48,49,50,51]. The reason for this is that surveyed participants most likely would belong to the poorest populations of the country with no significant variations in their socio-economic status and thus with no effect in the examined outcomes here. Therefore overall, despite the aforementioned potential limitations, this study represents one of the few quantitative, comprehensively analyzed studies on the epidemiology of helminth infections, anaemia and undernutrition in Rwanda covering a broad age range with an extension particularly in the adolescents' years.

In conclusion the results of this study suggest that low-intensity polyparasite infections are more prevalent in Northern Rwanda, relative to high intensity polyparasitism, at least in terms of the major species of parasites under focus in the current study, and such co-infections appear not to have, alone, a great impact on

anaemia and undernutrition. Consequently based on the current findings we would support the argument that sufficient chemotherapy programmes to prevent high infection intensities build up in these people even without achieving parasite eradications, should be promoted. Finally, as currently there is a move towards drugs for integrated NTDs, we would urge for similar analytical studies in order to fully evaluate risks and benefits of such initiatives in helminth endemic regions.

Supporting Information

Alternative Language Abstract S1 Translation of the abstract into French by MAD.

Found at: doi:10.1371/journal.pntd.0000517.s001 (0.03 MB DOC)

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Author Contributions

Conceived and designed the experiments: DM BK AK ER TK MHK OM MK VN. Performed the experiments: DM BK ER TK MHK OM MK VN. Analyzed the data: AK. Contributed reagents/materials/analysis tools: AF. Wrote the paper: DM AK. Critical review of and contribution to intellectual content of paper: DM BK ER TK MHK OM MK VN MAD JPW AF. Participated in data collection: DM BK ER TK MHK OM MK VN MAD AF. Drafted the first version of this paper: AK. Obtained the funding and was the principal investigator: JR.

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